

# Detection and Identification of Spotted Fever Group Rickettsiae and *Ehrlichiae* in African Ticks

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*Rickettsia africae*, a recently identified pathogen, was detected for the first time in *Amblyomma* ticks from Niger, Mali, Burundi, and Sudan, and “*R. mongolotimonae*” was identified for the first time in Africa. Rickettsiae of unknown pathogenicity and two new *Ehrlichiae* of the *Ehrlichia canis* group were identified in ticks from Mali and Niger.

Spotted fever group Rickettsiae and *Ehrlichiae* are obligate intracellular gram-negative bacteria associated with arthropods, mainly ticks. While feeding, ticks can transmit these microorganisms to humans and animals (1). Two human tick-borne rickettsioses are known to occur in Africa (2). Mediterranean spotted fever, caused by *Rickettsia conorii*, is transmitted by the brown dog tick, *Rhipicephalus sanguineus*, which is well adapted to urban environments. *R. conorii* is prevalent in the Mediterranean area (Tunisia, Algeria, Morocco, Libya, and Egypt) and has also been isolated or detected in Kenya, Central Africa, Zimbabwe, and South Africa (2). Although African tick bite fever has been recognized since the beginning of the century as a rural disease usually contracted from ticks of cattle and game, it was regarded as synonymous with Mediterranean spotted fever, until the first human infection with *R. africae* was reported from Zimbabwe in 1992. Subsequently, numerous cases have been reported in tourists returning from southern Africa, where the cattle tick *Amblyomma hebraeum* is the vector (2,3). *R. africae* has also been recovered from *A. variegatum* ticks in Ethiopia and central Africa (2). In 1992, a survey for antibodies against *Ehrlichia chaffeensis* (the agent of human monocytic ehrlichiosis) in human sera from eight African countries indicated that human ehrlichioses might occur on the continent (4), and subsequently a case (diagnosed by serology only) was reported from Mali (5). Recently, new molecular methods have enabled the development of useful, sensitive, and rapid tools to detect and identify tick-borne pathogens in arthropods, including ticks (6). In this work, we tested ticks from Africa for rickettsial and ehrlichial DNA using polymerase chain reaction (PCR) and sequence analysis of amplified products.

## Materials and Methods

Ticks were kept frozen at -20°C (in Niger) or at -80°C (in other countries) before being tested. DNA of each tick was extracted as described (7). Rickettsial and ehrlichial DNA was detected by PCR as described, using specific primers (Table).

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The sequences of PCR products were obtained and analyzed with the corresponding sequences of rickettsial or ehrlichial species as described (7). Multiple alignment analysis was performed by using the ClustalW program version 1.8 in the DNA Data Bank of Japan (DDBJ; Mishima, Japan [<http://www.ddbj.nig.ac.jp/htmls/E-mail/clustalw-e.html>]). All sequences used in the study are available in GenBank; the accession numbers of the new genotypes detected in this work are shown in the Table footnotes.

## Results

Rickettsial DNA was detected in 24 (7.2%) of the 332 ticks examined (Table). *R. africae* was detected from *A. variegatum* from Mali (2/6), Niger (2/6), and Burundi (1/13), and from 1 of 16 *A. lepidum* from the Sudan. *R. aeschlimannii* was detected in *Hyalomma marginatum rufipes* from Niger and Mali (8/24 and 3/20, respectively) and *R. massiliae* in 2/37 *Rh. muhsamae* from Mali. Further, three new *ompA* sequences (590 bp) were obtained from *A. variegatum* from Mali and Niger (Table). These were 99.3%–99.5% identical to those of *R. africae*. In the phylogenetic tree based on these *ompA* sequences, the three rickettsiae (named RAy1, RAy3, RAy9) were closely related to one another (95.7% bootstrap value) and branched with *R. africae* (86.1% bootstrap value) (data not shown). Partial sequences (316 bp) of the *gltA* gene of RAy1, RAy3, and RAy9 were also found to be closely related to those of *R. africae* (99% of similarity). Two new 16S rRNA ehrlichial genotypes were detected, including ERm58 (1,380 bp) in 7/37 *Rh. muhsamae* from Mali and EHt224 (1366 bp) in 1/5 *H. truncatum* from Niger. Both sequences were very similar (99.34% similarity), but different from those described for all the known ehrlichiae (i.e., 98.55% similarity with *E. chaffeensis*, 98.26% with *E. canis* and *E. ewingii*, and 97.75% with *E. muris* and *Cowdria ruminantium*). In a phylogenetic tree based on 16S rRNA gene sequences, ERm58 and EHt224 were found to be closely related and to belong to the *E. canis* group (data not shown). Enlarged *gltA* sequences of ERm58 (1,140 bp) and EHt224 (1,189 bp) were also obtained from the above ticks. Phylogenetic analyses of these sequences confirmed that ERm58 and EHt224 belonged to the *E. canis* group (data not shown).

## Research

Table. Detection and identification of spotted fever group *Rickettsiae* and *Ehrlichiae* from African ticks by polymerase chain reaction (PCR)<sup>a,b</sup>

Tick species	Animal	Location	No. pos. ticks/ total examined	Gene sequence	Identification	GenBank accession no. for new genotypes
<i>Amblyomma variegatum</i>	Cattle	Mali	6/6 (rickettsiae)	ompA	<i>Rickettsia africae</i> (2/6)	-
					RAv1 (2/6) genotype	AF311959
					RAv3 (2/6) genotype	AF311960
				gltA	<i>R. africae</i> (2/6)	-
		Niger	6/6 (rickettsiae)	ompA	RAv1 (2/6) genotype	AF311962
					RAv3 (2/6) genotype	AF311963
		Burundi	1/13 (rickettsiae)	ompA + gltA	<i>R. africae</i>	-
		Sudan	1/16 (rickettsiae)	ompA + gltA	<i>R. africae</i>	-
	Cattle	Niger	0/42	-	-	-
		Mauritania	0/42	-	-	-
<i>H. dromedarii</i>	Cattle	Niger	0/7	-	-	-
<i>H. impressum</i>	Cattle	Niger	0/8	-	-	-
<i>H. marginatum rufipes</i>	Cattle	Niger	8/24 (rickettsiae)	ompA + gltA	<i>R. aeschlimannii</i>	-
		Mali	3/20 (rickettsiae)	ompA + gltA	<i>R. aeschlimannii</i>	-
<i>H. truncatum</i>	Cattle	Niger	1/5 (rickettsiae)	ompA + gltA	<i>R. mongolimonae</i>	-
			1/5 (ehrlichiae)	16S rRNA gene	Eht224 genotype	AF311968
		Mali	0/5	gltA	-	-
		Sudan	0/5	ompA + gltA	-	-
				ompA + gltA	-	-
<i>Rhipicephalus muhsamae</i>	Cattle	Mali	2/37 (rickettsiae)	ompA + gltA	<i>R. massiliae</i>	-
			7/37 (ehrlichiae)	16S RNA gene	Erm58 genotype	AF311967
				gltA	Erm58 genotype	AF311965
<i>R. evertsi evertsi</i>	Cattle	Sudan	0/10	-	-	-
<i>R. sanguineus</i>	Dogs	Mali	0/24	-	-	-
		Sudan	0/62	-	-	-

<sup>a</sup>A convenience sample of ticks was obtained as part of other, ongoing studies, as summarized above. In October 1997, 42 *Hyalomma impeltatum* were collected from cattle at Kiffa (16°37'N, 11°24'O) in Mauritania. In Mali in February 1998, 6 *Amblyomma variegatum*, 37 *R. muhsamae*, 20 *H. marginatum rufipes*, and 5 *H. truncatum* were collected from cattle in Bamako (12°39' N, 8°00'W) and Bougouni (11°25' N, 7°29' W) and 24 *R. sanguineus* from dogs in Bamako. In 1999, 6 *A. variegatum*, 42 *H. impeltatum*, 7 *H. dromedarii*, 8 *H. impressum*, 24 *H. marginatum rufipes*, and 5 *H. truncatum* were collected from cattle at Niamey (13°30' N, 2°07' E) in the Republic of Niger; 5 *H. truncatum*, 10 *Rh. evertsi evertsi*, and 16 *A. lepidum* were collected from cattle and 62 *Rh. sanguineus* were collected from dogs in Khartoum (15°31' N, 32°47' E) in the Sudan; 13 *A. variegatum* were collected from cattle in Bujumbura (3°22' S, 29°21' E) in Burundi. All ticks were adults attached on mammals.

<sup>b</sup>Primers include Rr190.70p and Rr190.701n, which amplify a fragment of 629-632 bp of *ompA* encoding for a 190-kD protein (7), and RpCS.877p-RpCS.1273r, which amplify a 396-bp fragment of the citrate synthase gene, *gltA* (7). Ehrlichial DNA was detected with EHR16SR-EHR16SD primers, which amplify a 345-bp fragment of the 16S rRNA gene of all the known ehrlichiae (8). To amplify the main part of the 16S rRNA gene, tick DNA samples that were found to be positive with the above primers were amplified with the EHR16SR and EHR16SD primers and the universal primers fD1 and rp2 (7). The positive DNA samples were also used in PCR reactions to amplify the citrate synthase gene, *gltA*, of *Ehrlichiae*. A set of primers, EHR-CS133F (5'-GGW-TTY-ATG-TCY-ACT-GCT-GC-3') and EHR-CS778R (5'-GCN-CCM-CCA-TGM-GCT-GG-3'), which amplify a fragment of about 650 bp of the citrate synthase gene of tick-borne *Ehrlichiae*, were used for the screening PCR. Two other primer sets, Chaff-M4F (5'-AAT-TAT-GRT-YAA-ARA-RGC-AG-3')/EHR-CS778R and F1b(5'-GAT-CAT-GAR-CAR-AAT-GCT-TC-3')/Chaff1233R (5'-ACC-AGT-ATA-YAA-YTG-ACG-3') were used to amplify the main part of the citrate synthase gene sequence in tick DNA samples that were found to be positive from the screening PCR (Inokuma, et al., unpub data).

## Conclusion

This study has shown for the first time that *R. africae*, the agent of African tick bite fever, is present in West Africa (Mali and Niger), the Sudan, and Burundi. It also indicates a potential role for *A. variegatum* and *A. lepidum* as vectors of *R. africae* in these areas. Recently, we also documented cases in tourists returning from numerous countries, including those in West and East Africa (9) (Figure). Our results support the hypothesis that the geographic distribution of African tick bite fever parallels that of the distribution of *Amblyomma* spp., as ticks are known to be vectors and also reservoirs of tick-borne rickettsiae (2). In Africa, although the principal vector of *R. africae* appeared to be *A. hebraicum*, which is prevalent in southern Africa, *A. variegatum* (which is widely distributed

throughout sub-Saharan Africa) appears as a potential vector. *Amblyomma* are known to readily feed on people in Africa and are commonly infected with rickettsiae (up to 100%). Thus, African tick bite fever may have a high prevalence throughout the continent. Studies have shown seroprevalences of 30%–80% for spotted fever group rickettsiae in sub-Saharan Africa (2), although it is unclear what proportion of those infections might be due to *R. africae* infection.

In this study, we report for the first time the presence of "*R. mongolotimonae*" in Africa (Niger). This pathogen was first isolated from an *H. asiaticum* collected in Inner Mongolia, China. Later, the same agent was isolated from the blood and skin of a febrile woman from Marseille in 1996 (2), which demonstrated its pathogenicity for humans. Subsequently, we have recognized four more cases in southern France (10, and unpub. data). Results of this study suggest that *R. mongolotimonae* may be associated with *Hyalomma* sp. ticks throughout the world. In this work, we also detected two rickettsiae of unknown pathogenicity, namely *R. aeschlimannii* and *R. massiliæ*. Although this is the first recognition of these rickettsiae in Mali and Niger, the epidemiologic importance of this finding has yet to be determined. Finally, we detected three new spotted fever group genotypes closely related to *R. africae*. Until further studies clarify the position of these organisms, we suggest they may be considered variant strains of *R. africae*.

Although previous reports, based on the results of serosurveys, have indicated that human ehrlichioses occur in Africa, firm evidence is still absent. Because of the serologic cross-reactivity between ehrlichiae, serosurvey results have to be interpreted carefully. We detected two new ehrlichial genotypes, that is, Erm58 in *Rh. muhsamae* from Mali and Eht224 in *H. truncatum* from Niger. Both belong to the *E. canis* group, which includes *E. chaffeensis*, *C. ruminantium*, *E. muris*, *E. ewingii*, and a new isolate detected in Japanese ticks (11). In this group, as within each group of ehrlichiae, members share homologous surface antigens and thus cross-

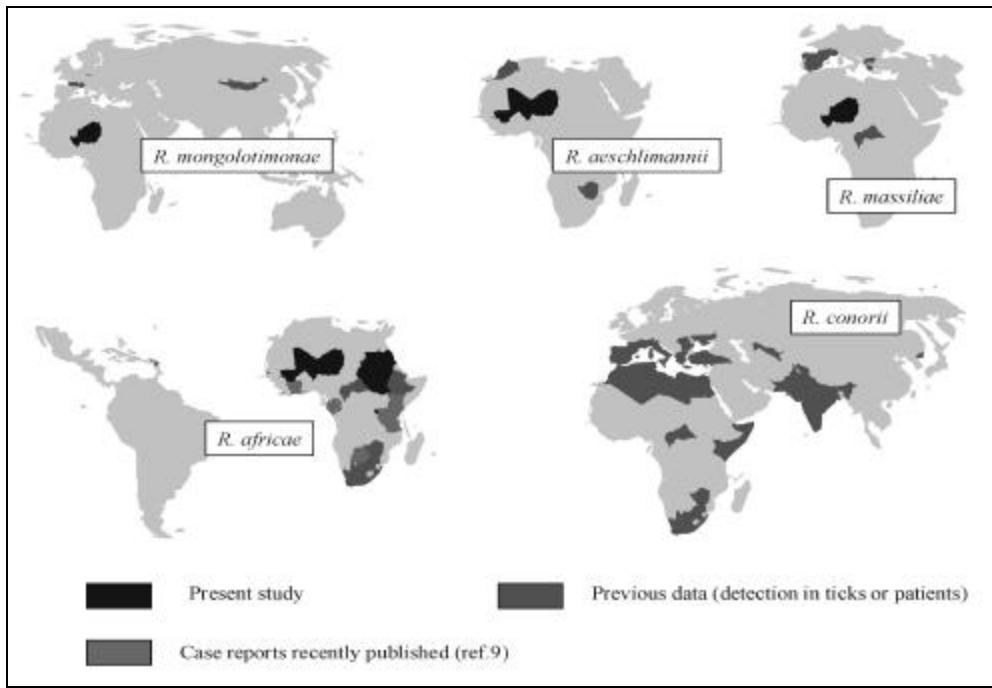


Figure. Geographic distribution of spotted fever group Rickettsiae occurring in Africa. R. = *Rickettsia*.

react extensively in serologic assays (12). Erm58 and Eht224 may also be organisms responsible for such serologic cross-reactions, including in serosurveys and case reports of human ehrlichioses in Africa. In 1997, new ehrlichial genotypes were also detected in Namibia and Zimbabwe, and a number of ehrlichiae in Africa may be responsible for serologic cross-reactions in serosurveys of humans and animals for currently recognized pathogenic ehrlichiae (13,14). The pathogenicity of the Erm58 and Eht224 recognized in our study has yet to be determined, and further studies to characterize the human ehrlichioses in Africa are indicated. Moreover, it remains to be demonstrated whether *H. truncatum* and *Rh. muhsamae* ticks act as vectors or reservoirs of the new ehrlichiae, since ticks also could have been infected while feeding on bacteremic mammals.

Although this study detected for the first time certain rickettsiae and ehrlichiae in African countries, systematic sampling was not done, and results cannot address their prevalence and distribution. However, this work provides a starting point for epidemiologic studies there.

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